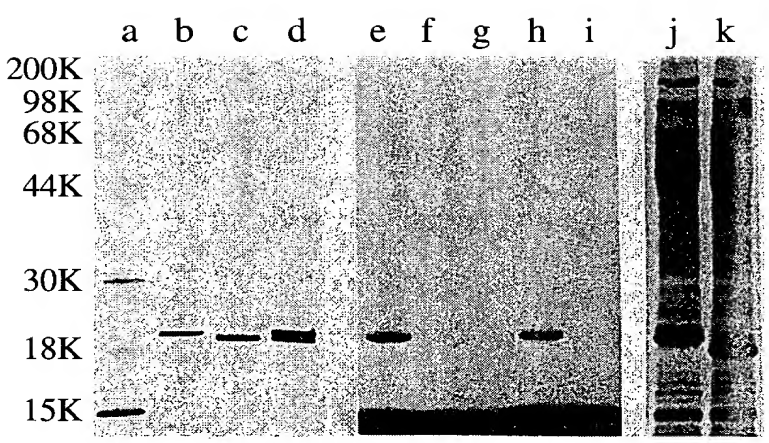

Fig. 1

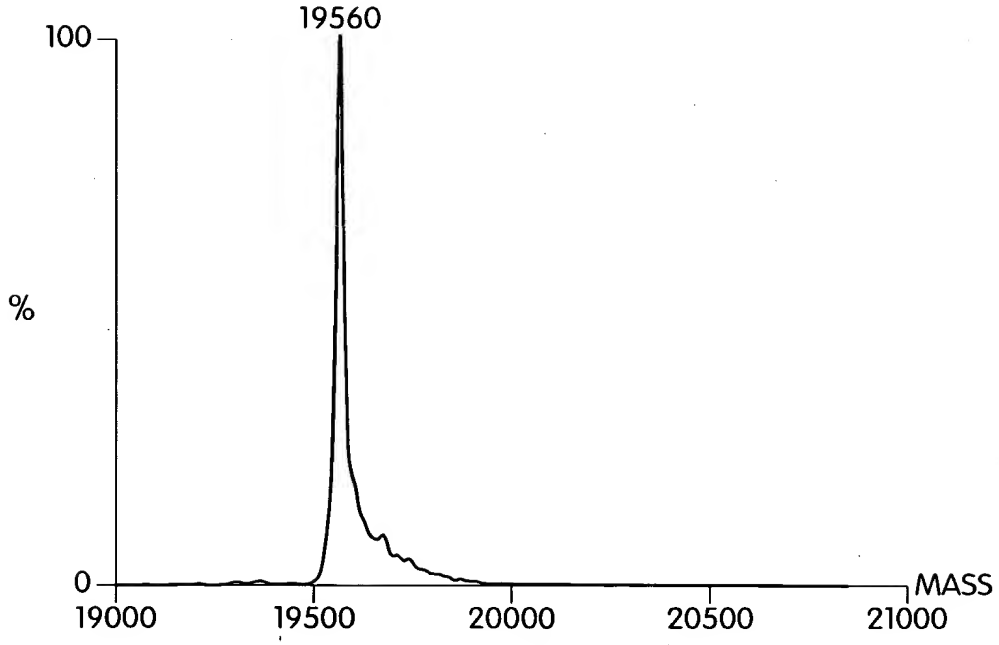
19560
100
%
0
MASS
19000
19500
20000
20500
21000

Fig. 2A

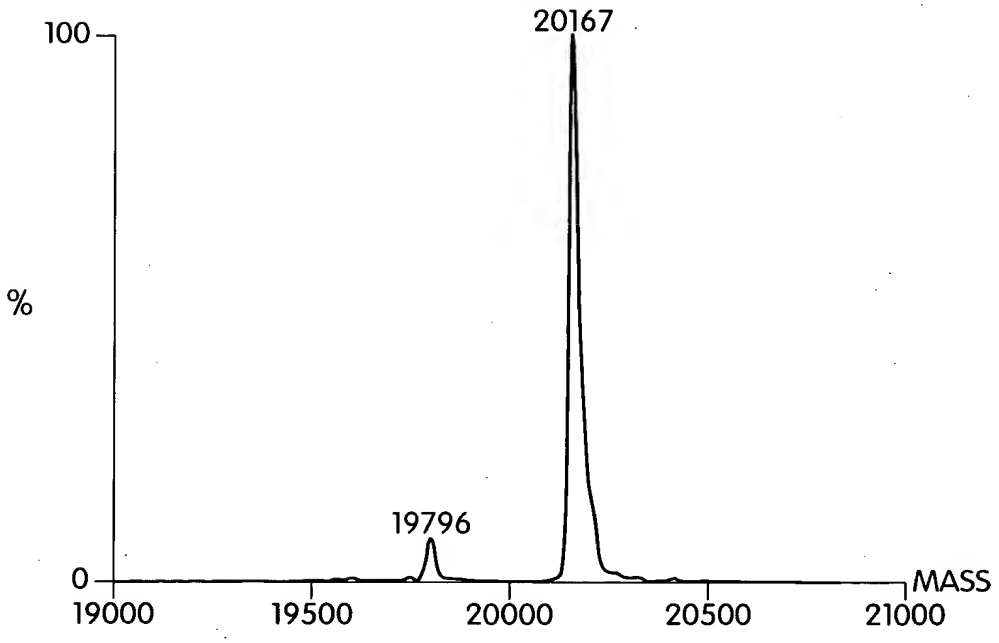
20167
100
%
19796
0
MASS
19000
19500
20000
20500
21000

Fig. 2B

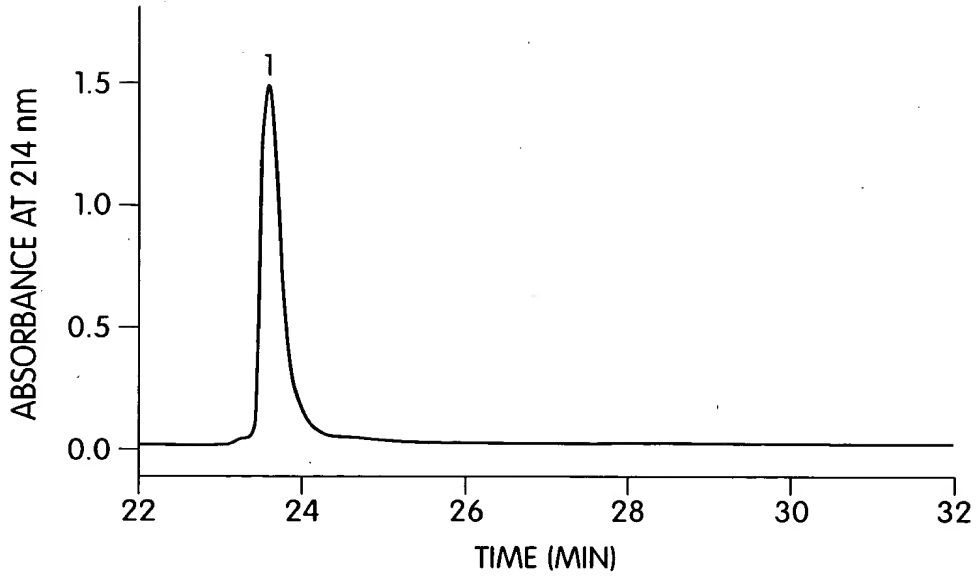
ABSORBANCE AT 214 nm
1.5
1.0
0.5
0.0
1
22
24
26
28
30
32
TIME (MIN)

Fig. 3A

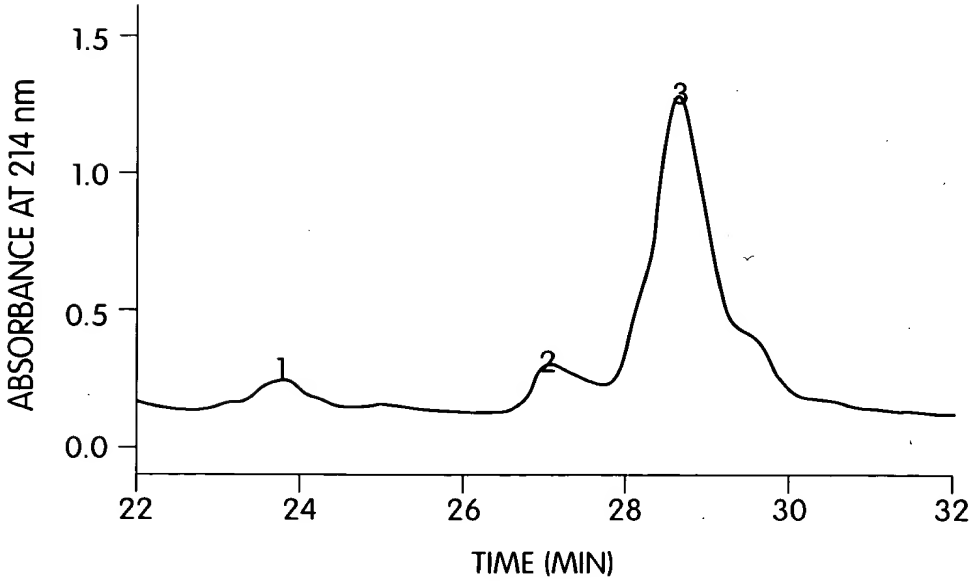
ABSORBANCE AT 214 nm
1.5
1.0
0.5
0.0
1
2
3
22
24
26
28
30
32
TIME (MIN)

Fig. 3B

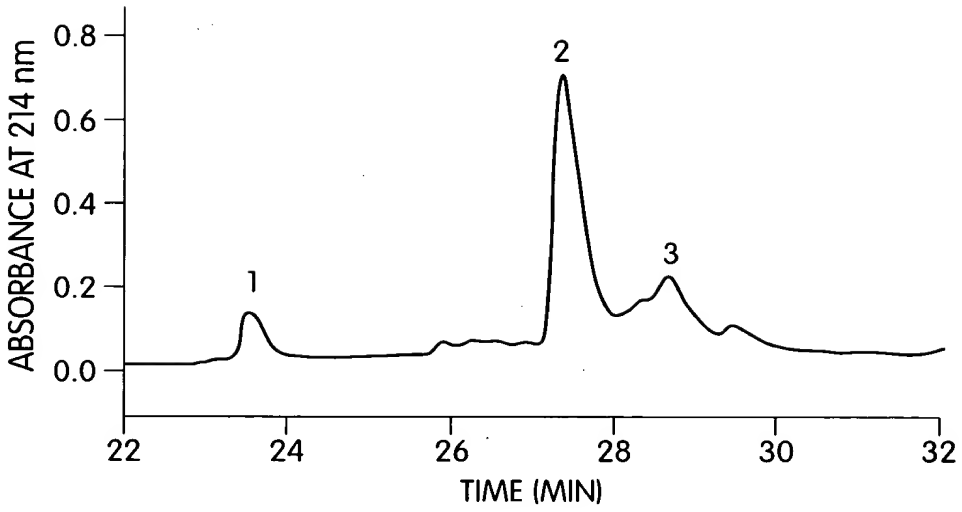
ABSORBANCE AT 214 nm
0.8
0.6
0.4
0.2
0.0
1
2
3
22
24
26
28
30
32
TIME (MIN)

Fig. 3C

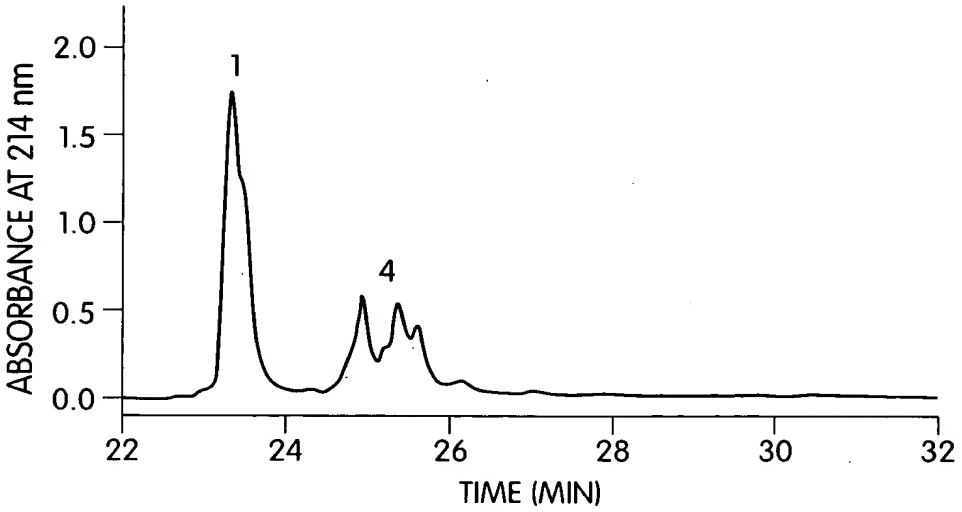
ABSORBANCE AT 214 nm
2.0
1.5
1.0
0.5
0.0
1
4
22
24
26
28
30
32
TIME (MIN)

Fig. 3D

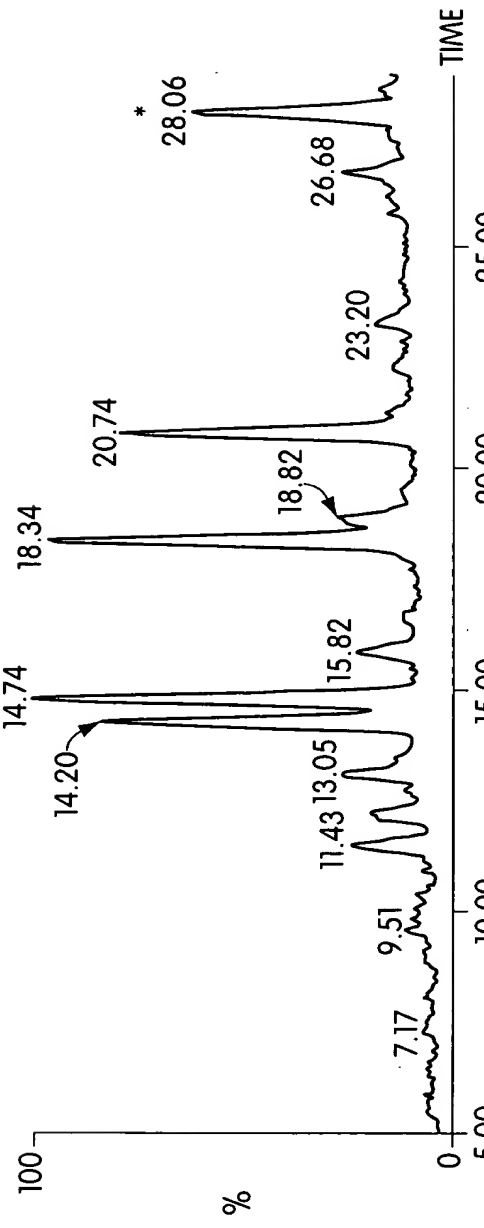
100
%
0
5.00
10.00
15.00
20.00
25.00
TIME
7.17
9.51
11.43
13.05
14.20
14.74
15.82
18.34
18.82
20.74
23.20
26.68
*
28.06

Fig. 4A

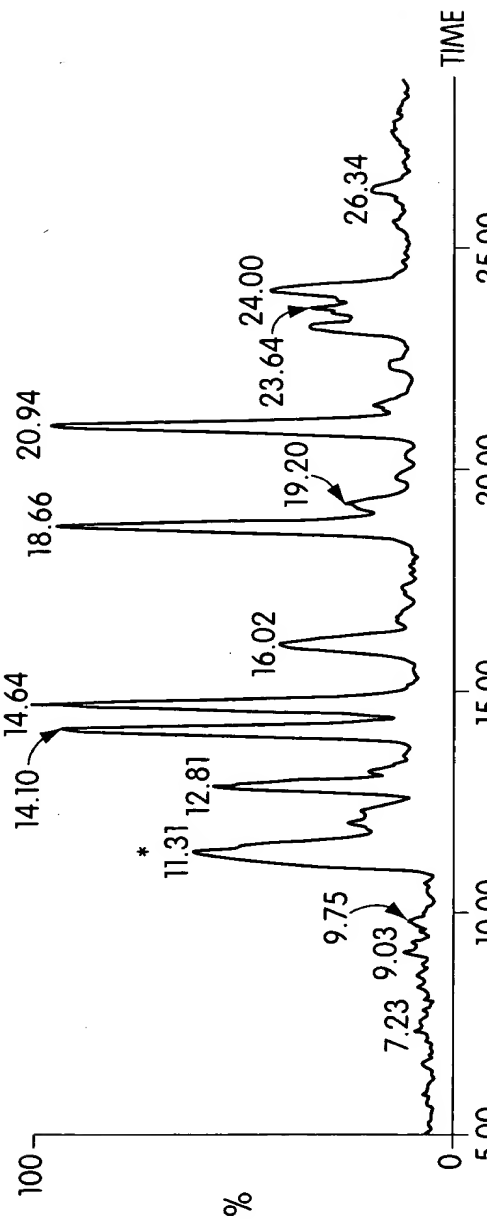
100
%
0
5.00
10.00
15.00
20.00
25.00
TIME
7.23
9.03
9.75
*
11.31
12.81
14.10
14.64
16.02
18.66
19.20
20.94
23.64
24.00
26.34

Fig. 4B

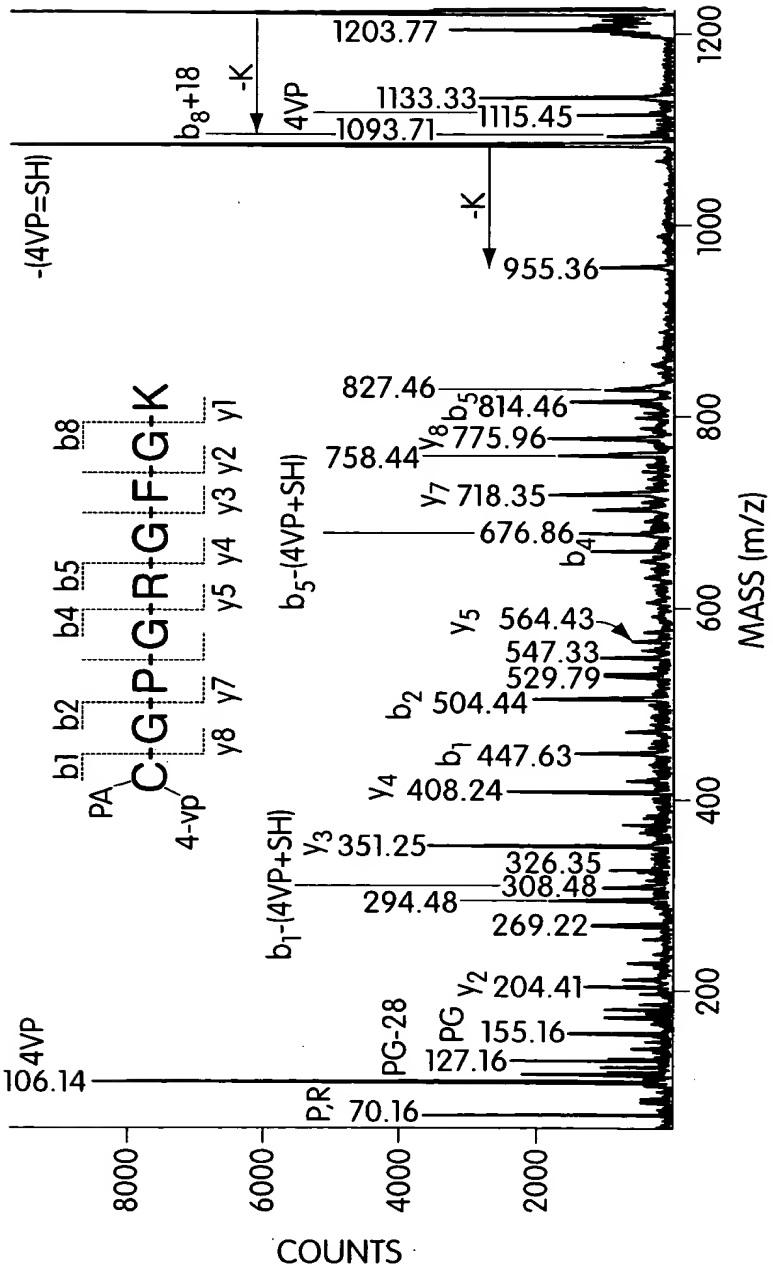
1203.77
1133.33
1115.45
1093.71
955.36
827.46
814.46
775.96
758.44
718.35
676.86
564.43
547.33
529.79
504.44
447.63
408.24
351.25
326.35
308.48
294.48
269.22
204.41
155.16
127.16
106.14
70.16
b8+18
-K
4VP
-(4VP=SH)
b5
y8
y7
b4
y5
b5-(4VP+SH)
b2
b1
y4
y3
b1-(4VP+SH)
y2
PG
PG-28
P,R
1200
1000
800
600
400
200
MASS (m/z)
8000
6000
4000
2000
COUNTS
b8
b5
y1
y2
y3
y4
y5
y7
y8
b1
b2
b4
PA
C-G-P-G-R-G-F-G-K
4-vp

Fig. 5

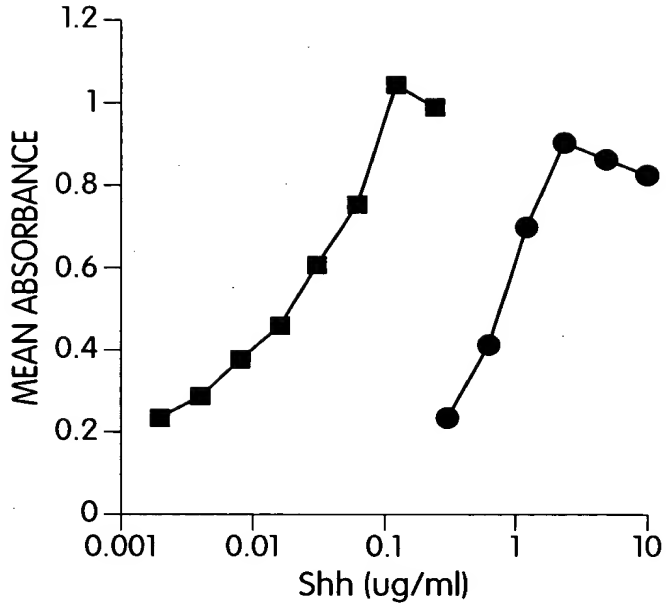
1.2
1
0.8
0.6
0.4
0.2
0
MEAN ABSORBANCE
0.001
0.01
0.1
1
10
Shh (ug/ml)

Fig. 6A

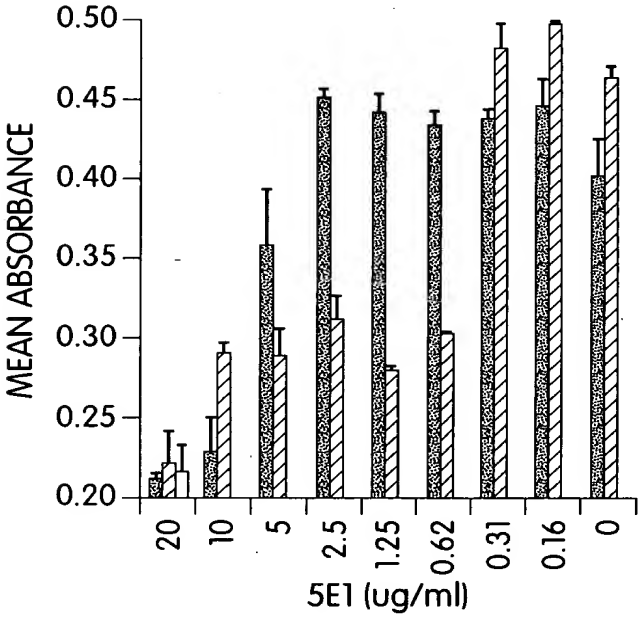
0.50
0.45
0.40
0.35
0.30
0.25
0.20
MEAN ABSORBANCE
20
10
5
2.5
1.25
0.62
0.31
0.16
0
5E1 (ug/ml)

Fig. 6B

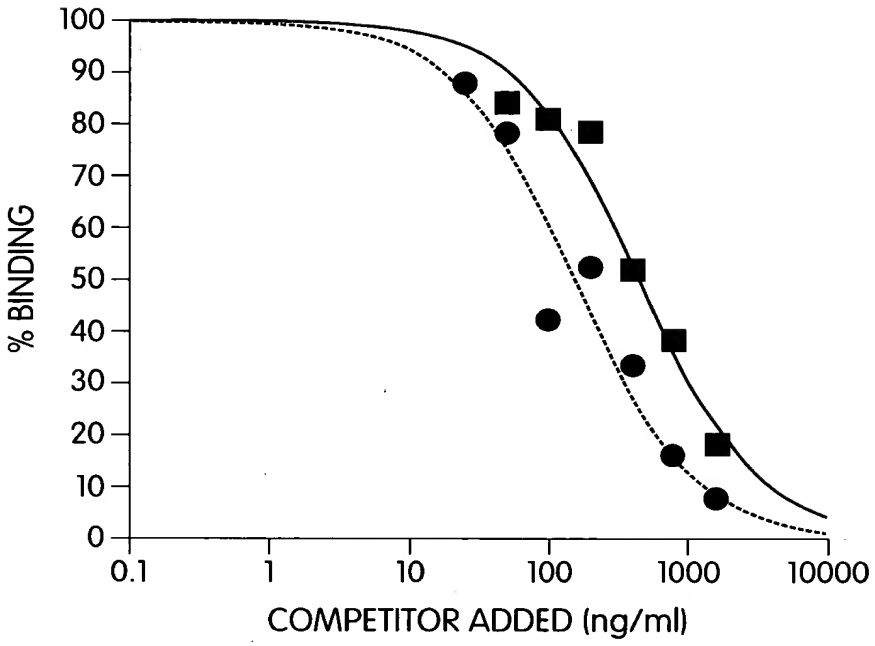
100
90
80
70
60
50
40
30
20
10
0
% BINDING
0.1
1
10
100
1000
10000
COMPETITOR ADDED (ng/ml)

Fig. 7

```
       1                                                            50
Indian CGPGRVVGSR RRPPRK-LVP LAYKQFSPNV PEKTLGASGR YEGKIARSSE
Sonic  CGPGRGFG-K RRHPKK-LTP LAYKQFIPNV AEKTLGASGR YEGKISRNSE
Desert CGPGRGPVGR RRYARKQLVP LLYKQFVPGV PERTLGASGP AEGRVARGSE

       51                                                           100
Indian RFKELTPNYN PDIIFKDEEN TGADRLMTQR CKDRLNSLAI SVMNQWPGVK
Sonic  RFKELTPNYN PDIIFKDEEN TGADRLMTQR CKDKLNALAI SVMNQWPGVK
Desert RFRDLVPNYN PDIIFKDEEN SGADRLMTER CKERVNALAI AVMNMWPGVR

       101                                                          150
Indian LRVTEGWDED GHHSEESLHY EGRAVDITTS DRDRNKYGLL ARLAVEAGFD
Sonic  LRVTEGWDED GHHSEESLHY EGRAVDITTS DRDRSKYGML ARLAVEAGFD
Desert LRVTEGWDED GHHAQDSLHY EGRALDITTS DRDRNKYGLL ARLAVEAGFD

       151                      176
Indian WVYYESKAHV HCSVKSEHSA AAKTGG     SEQ ID NO: 1
Sonic  WVYYESKAHI HCSVKAENSV AAKSGG     SEQ ID NO. 2
Desert WVYYESRNHV HVSVKADNSL AVRAGG     SEQ ID NO. 3
```

Gap(s), indicated by -, added to facilitate alignment

Fig. 8

1 40
CGPGR$_{X1\ X2\ X3\ X4\ X5}$ RR$_{X6\ X7\ X8}$K$_{X9}$L$_{X10}$P L$_{x11}$YKQF$_{X12}$P$_{X13}$V $_{X14}$EKTLGASGR

80
$_{X15}$EGK$_{X16\ X17}$R$_{X18}$SE RFK$_{X19}$L$_{X20}$PNYN PDIIFKDEEN $_{X21}$GADRLMT$_{x22}$R

120
CK$_{X23\ X24X25}$NSLAI $_{X26}$VMN$_{X27}$WPGVK LRVTEGWDED GHH$_{X28\ X29\ X30}$SLHY

160
EGRAVDITTS DRDR$_{X31}$KYG$_{X32}$L ARLAVEAGFD WVYYES$_{X33\ X34}$H$_{X35}$

176
H$_{X36}$SVK$_{X37\ X38\ X39}$S$_{X40}$ AA$_{X41\ X42}$GG

Where:
X1 is either V or G;
X2 is either V, F or P;
X3 is either G or V;
X4 is either S or G;
X5 is either R or K;
X6 is either P, H or Y;
X7 is either P or A;
X8 is either R or K;
X9 is any amino acid;
X10 is either V or T;
X11 is either A or L;
X12 is either S, I or V;
X13 is either N or G ;
X14 is either P or A;
X15 is either Y or A;
X16 is either I or V;
X17 is either A or S;
X18 is either S, N or G;
X19 is either E or D;
X20 is either T or V;
X21 is either T or S;
X22 is either Q or E;
X23 is either D or E;
X24 is either R or K;
X25 is either L or V;
X26 is either S or A;
X27 is either Q or M;
X28 is either S or A;
X29 is either E or Q;
X30 is either E or D;
X31 is either N or S;
X32 is either L or M;
X33 is either K or R;
X34 is either A or N;
X35 is either V or I;
X36 is either C or V;
X37 is either S or A;
X38 is either E or D;
X39 is either H or N;
X40 is either A, V or L;
X41 is either K or R; and
X42 is either T, S or A.

Fig. 9

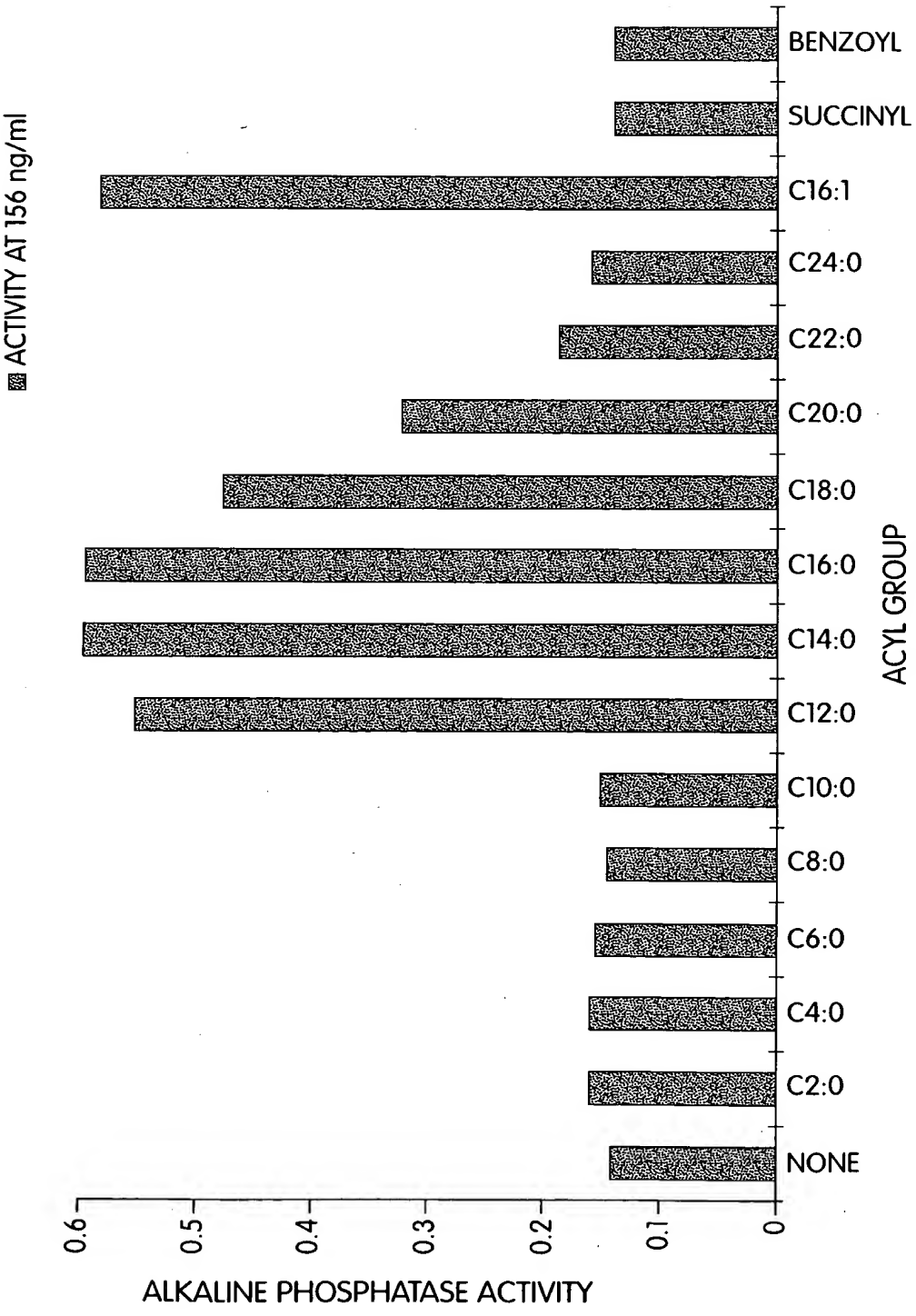
ACTIVITY AT 156 ng/ml
BENZOYL
SUCCINYL
C16:1
C24:0
C22:0
C20:0
C18:0
C16:0
C14:0
C12:0
C10:0
C8:0
C6:0
C4:0
C2:0
NONE
ACYL GROUP
0.6
0.5
0.4
0.3
0.2
0.1
0
ALKALINE PHOSPHATASE ACTIVITY

Fig. 10

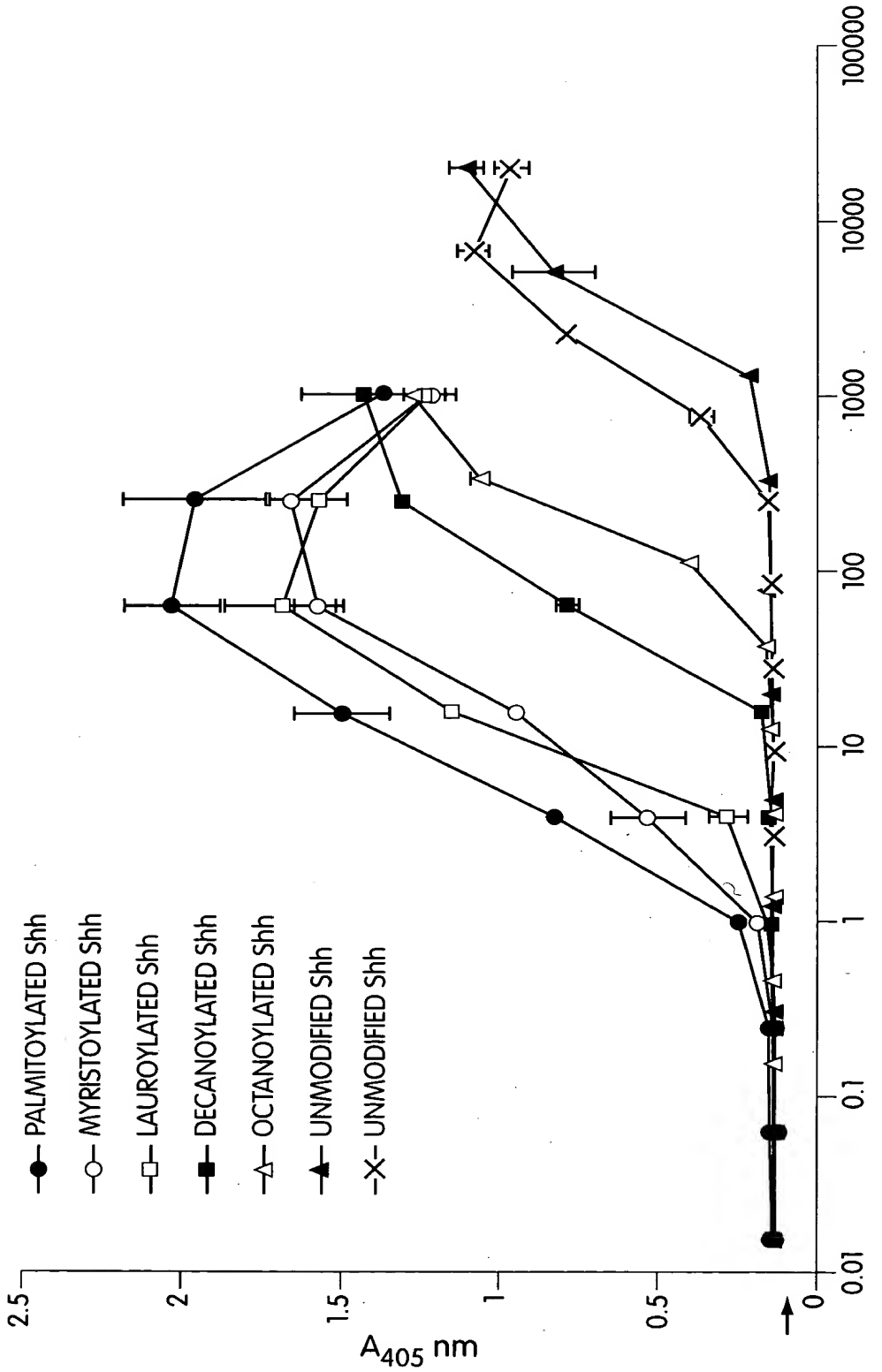
PALMITOYLATED Shh
MYRISTOYLATED Shh
LAUROYLATED Shh
DECANOYLATED Shh
OCTANOYLATED Shh
UNMODIFIED Shh
UNMODIFIED Shh
A405 nm
2.5
2
1.5
1
0.5
0
0.01
0.1
1
10
100
1000
10000
100000
[PROTEIN] (ng/ml)

Fig. 11

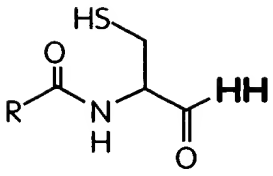

Fig. 12A

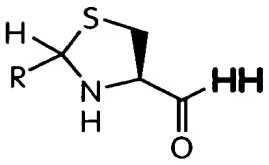

Fig. 12B

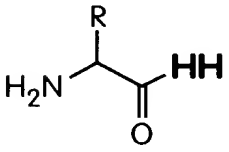

Fig. 12C

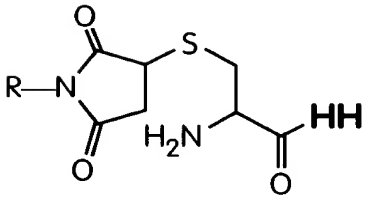

Fig. 12D

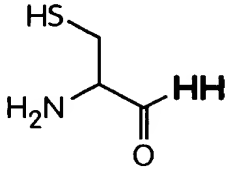

Fig. 12E

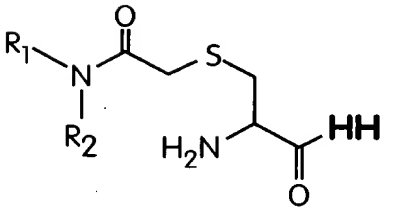

Fig. 12F

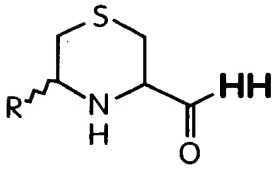

Fig. 12G

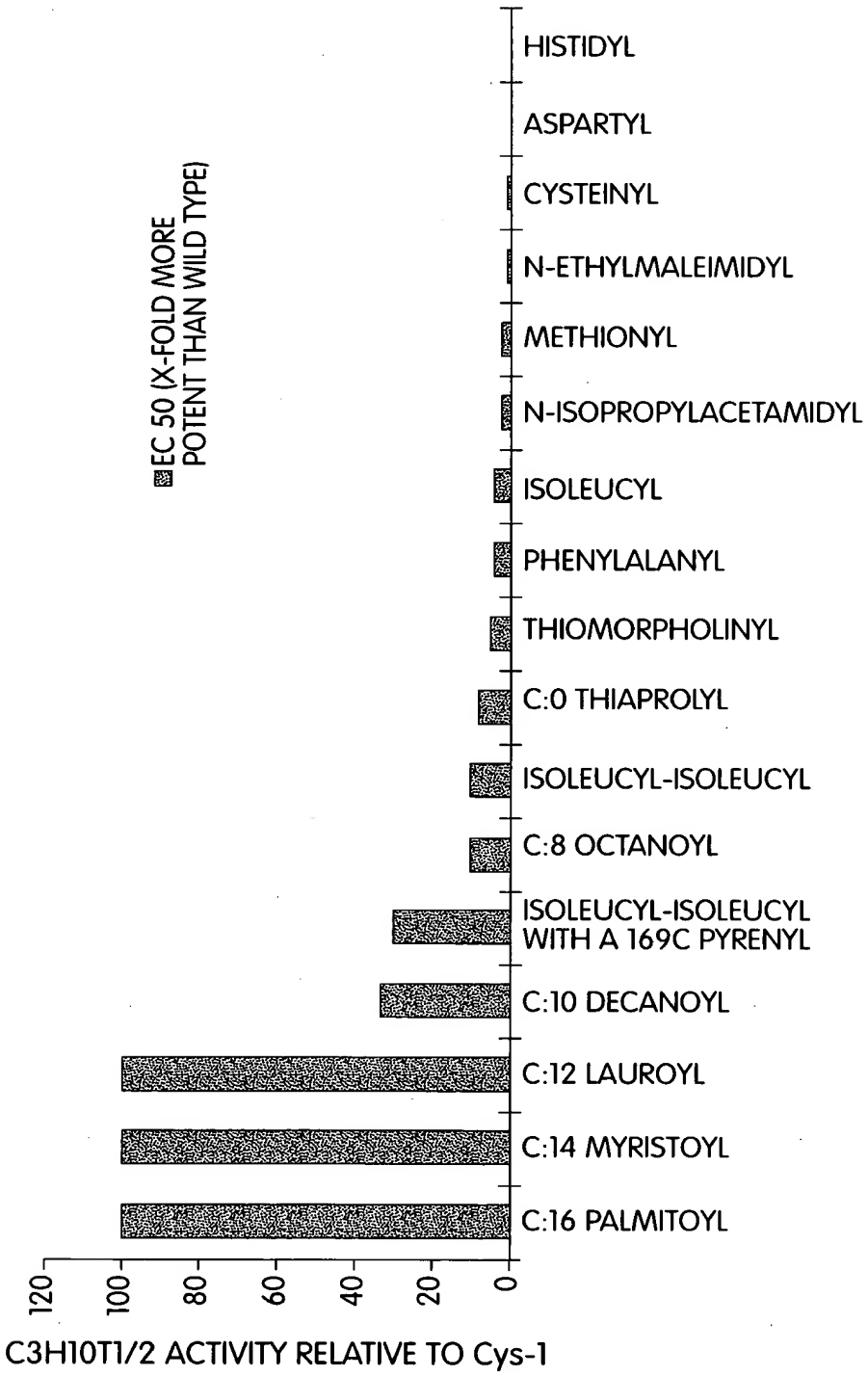
EC 50 (X-FOLD MORE POTENT THAN WILD TYPE)
HISTIDYL
ASPARTYL
CYSTEINYL
N-ETHYLMALEIMIDYL
METHIONYL
N-ISOPROPYLACETAMIDYL
ISOLEUCYL
PHENYLALANYL
THIOMORPHOLINYL
C:0 THIAPROLYL
ISOLEUCYL-ISOLEUCYL
C:8 OCTANOYL
ISOLEUCYL-ISOLEUCYL WITH A 169C PYRENYL
C:10 DECANOYL
C:12 LAUROYL
C:14 MYRISTOYL
C:16 PALMITOYL
120
100
80
60
40
20
0
C3H10T1/2 ACTIVITY RELATIVE TO Cys-1
MODIFICATION

Fig. 13

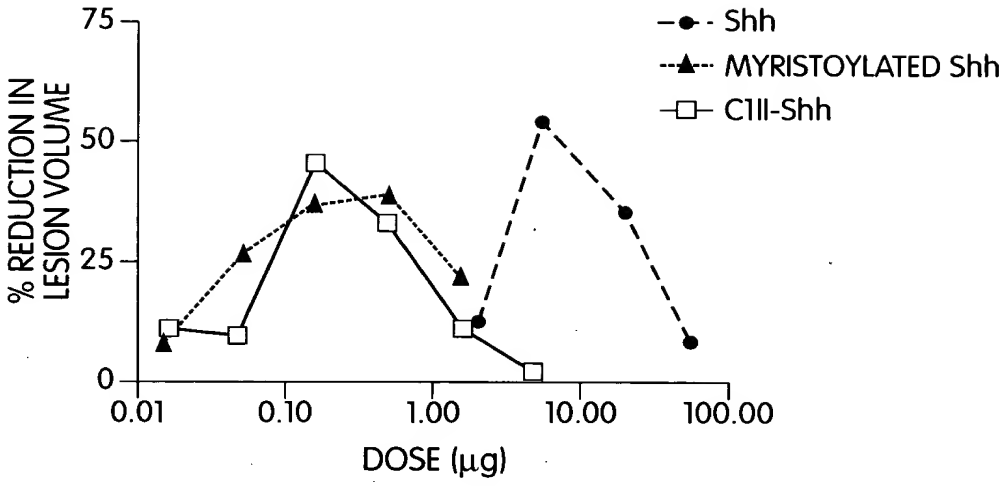
75
50
25
0
% REDUCTION IN LESION VOLUME
0.01
0.10
1.00
10.00
100.00
DOSE (µg)
Shh
MYRISTOYLATED Shh
C1II-Shh

Fig. 14

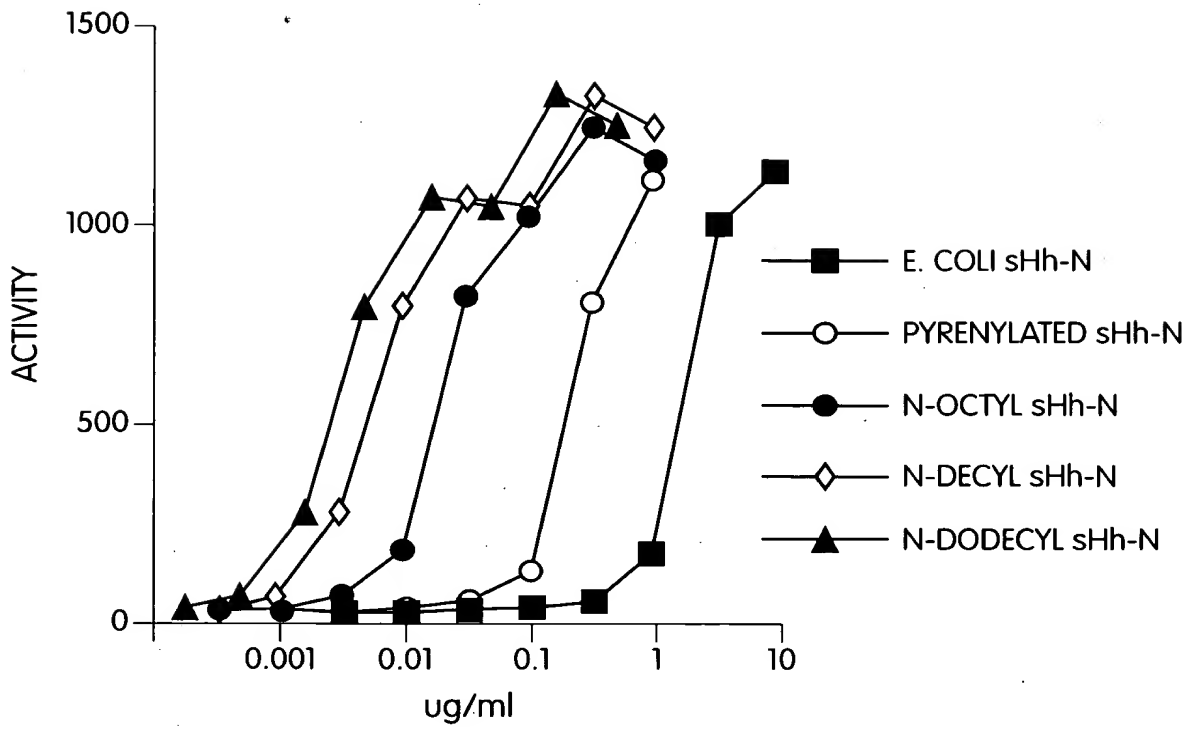
1500
1000
500
0
ACTIVITY
0.001
0.01
0.1
1
10
ug/ml
E. COLI sHh-N
PYRENYLATED sHh-N
N-OCTYL sHh-N
N-DECYL sHh-N
N-DODECYL sHh-N

Fig. 15